REMARKS/ARGUMENTS

Claims 23 and 35 are amended. Support for amended claims 23 and 35 can be found, e.g., at page 9, lines 4-7 of the specification. No new matter is introduced. Claims 1-42 are pending in the application. Reexamination and reconsideration of the application, as amended, are respectfully requested.

Claim Rejection Under 35 U.S.C. § 112, First Paragraph

Claims 1-22, 26-30, 32-34, 37-38, and 41-42 are rejected as failing to comply with the enablement requirement. See the Office Action, pages 3-8, part 5. The Examiner rejected these claim for various reasons, each of which is respectfully traversed as follows:

(1) Determining analyte mass by measuring fluorescence

The Examiner asserted that the specification does not teach a method of determining analyte mass by measuring fluorescence. Specifically, the Examiner asserted that "the art analyte mass detection is highly unpredictable and the instant specification fails to provide any information that the mass of any analyte could be detected in the claimed manner." The Examiner appears to believe that analyte mass can only be determined by resolving mathematical equations of mass action law. The Examiner thus asserted that the specification fails to provide protocols and actual data demonstrating how to apply these equations to determine analyte mass from fluorescence measurement. Applicants respectfully disagree.

Contrary to the Examiner's assertion, the specification provides ample information that analyte mass can be determined using the claimed method. For instance, claim 1 is drawn to a binding assay for sensing analyte mass in a liquid sample. The method involves:

a) immobilizing an array on a surface of a substrate, wherein the array comprises a plurality of microscopic sorbent zones, wherein a microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner, the matrix extending up to 200 nm vertically from the surface of the substrate;

b) contacting a defined volume of sample believed to contain an analyte with at least one microscopic sorbent zone, the analyte binding partner in the microscopic sorbent zone being present in excess relative to the analyte, so that any analyte present in the defined volume is substantially depleted from the sample and concentrated on the microscopic sorbent zone to form an analyte capture complex with the analyte binding partner;

c) tagging the analyte capture complex with a fluorescent label;

d) illuminating the microscopic sorbent zone with a laser in the absence of liquid; and

e) detecting fluorescence emissions from any microscopic sorbent zone having an analyte capture complex tagged with a fluorescent label, thereby determining the analyte mass harvested from the defined volume of sample.

Three examples are provided in the specification to demonstrate this method (page 15, line 2 – page 23, line 32). For instance, in Example I, arrays of immobilized avidin spots were prepared by printing a solution of 1 mg/ml NeutrAvidin in buffered solution. The spots were contacted with varying volumes and concentrations of DBCY5-biotin to form avidin-biotin complexes. After washing and drying, the arrays were imaged and fluorescence from DBCY5 was measured for each spot (page 17, line 18 – page 18, line 11). When different volumes of DBCY5-Biotin having the same concentration were added to the avidin spots, the fluorescence reading changed with the volume (FIG. 4). In contrast, when different volumes of DBCY5-Biotin having the same number of molecules were added to the avidin spots, the fluorescence readings were consistent regardless of the varying volumes (FIG. 5A). This indicates that the printed avidin arrays responded to DBCY5-biotin mass, not concentration, because they had sufficient affinity and binding capacity to significantly deplete the solution of dye-biotin as the solution volume was reduced (page 20, lines 7-16 of the specification). As a result, the fluorescence reflected the analyte mass harvested from the defined volume of sample, i.e., the analyte mass originally present in the defined sample volume. A dose response curve was established by plotting the fluorescence readings versus

the numbers of DBCY5-biotin molecules in 200 ul (FIG. 5B). Such dose response curves can be used to determine DBCY5-biotin masses in test samples as discussed in detail below.

Contrary to the Examiner's apparent belief that analyte mass can only be determined by resolving mathematical equations of mass action law, analyte mass can also be determined using a standard dose response curve. It is well known in the art that a standard curve is a quantitative research tool. For instance, in Example 2 of EP 0 304 202 B1 ("'202"), cited by the Examiner, one anti-TNF antibody labeled with Texas Red was added to wells in a microtiter plate. Various standard solutions containing known concentrations of TNF and a test sample were added to the wells to form TNF-antibody complexes. Another anti-TNF antibody labeled with FITC was then added to the wells to bind the complexes. Fluorescence ratios (FITC/Texas Red) were obtained for each spot. A dose response curve was built up using the fluorescence ratios obtained from the standard solutions. The TNF concentration corresponding to the fluorescence ratio obtained from the test sample was determined according to the dose response curve. This concentration was the TNF concentration in the test sample. See page 8, lines 1-48 of the '202 patent. The same principle is applicable to the present invention. For instance, to determine the number of DBCY5-biotin molecules in a test sample, one skilled in the art can run 200 ul test sample through the protocol described in Example I of the specification (outlined above) and obtain a fluorescence reading. The number of DBCY5-biotin molecules corresponding to the fluorescence reading can be determined according to the established dose response curve (FIG. 5B of the specification). This number is the number of DBCY5-biotin molecules in the 200 ul test sample.

Therefore, contrary to the Examiner's assertion, the specification teaches a method of determining analyte mass by measuring fluorescence. In this connection, Applicants point out that "[t]he law does not require a specification to be a blueprint in order to satisfy the enablement requirement" (*Staehlin v. Secher*, 24 U.S.P.Q. 2d 11513, 1516 (Bd. Pat. App. & Int. 1992)) and that a specification need not describe –

and best omits – that which is well-known in the art (*In re Buchner*, 929 F.2d 660, 661, 18 U.S.P.Q. 2d 1331, 1332 (Fed. Cir. 1991)). Here, quantification using a standard curve is a well-known and commonly employed analytical method, as demonstrated by Example 2 of the '202 patent. In addition, the specification provides specific examples for establishing fluorescence – analyte mass dose response curves. Given the general knowledge and the teaching of the specification, one skilled in the art would be able to determine analyte mass in a test sample by measuring fluorescence. No undue experimentation is required.

(2) Assay conditions

The Examiner further asserted that neither the claims nor the specification recite the necessary equilibrium conditions and ambient conditions to perform the method for determining analyte mass. Applicants disagree.

Contrary to the Examiner's assertion, both the claims and the specification recite conditions for determining analyte mass. For example, claim 1 recites in step b) "the analyte binding partner in the microscopic sorbent zone being present in excess relative to the analyte, so that any analyte present in the defined volume is substantially depleted from the sample and concentrated on the microscopic sorbent zone to form an analyte capture complex with the analyte binding partner." This condition is also recited in the specification, e.g., at page 3, lines 29-31. In addition, the specification provides further guidance for optimizing the conditions: (1) utilizing sorbent materials having high affinity constants, e.g., $K_A>10^{10}$ liter/mole, to give stronger binding; (2) minimizing the sample volume; and (3) utilizing a large initial mass of analyte binding partner as a capture reagent (page 14, lines 5-9). Moreover, specific examples are given in the specification to demonstrate the conditions. For instance, at page 4, line 31 – page 5, line 1, the specification provides: "The assay can be optimized using a defined volume of sample from about 50 ul to about 500 ul. Preferably, the amount of the analyte binding partner immobilized in a sorbent zone is from 10^5 to about 10^{12} molecules of analyte binding partner and the diameter of the sorbent zones is about 60 um to about 500 um. Under these conditions, about 10^5 to about 10^{10} molecules of analyte can be detected

per sorbent zone." At page 10, lines 8-11, the specification provides: "The antibodies used will generally have conventional affinity constants on the order of about 10^8 to about 10^{11} liters/mole, however high affinity antibodies, having affinity constants of about 10^{12} to about 10^{13} can also be used."

Taken together, the claims and the specification recite sufficient assay conditions. One skilled in the art would be able to follow the guidance to practice the claimed method for determining analyte mass.

(3) Non-covalent immobilization of analyte binding partners

The claims are drawn to binding assays involving both non-covalent and covalent immobilization of analyte binding partners to a substrate. The Examiner asserted that, because the specification clearly states that the results from non-covalent immobilization have been abandoned, it is unclear that one skilled in the art could follow the general guidelines to achieve mass-sensing analysis. Applicants disagree.

The specification teaches both non-covalent and covalent immobilization of analyte binding partners. For instance, at page 13, lines 17-21, the specification provides: "The array of sorbent zones of the present invention can be created by simple pipetting, by jet printing or photolithography. Although photolinking the sorbent materials th[r]ough a mask may prove to be a superior method of fabricating an array within narrow tolerances, at present thermal/piezo jet printing is the method of choice for localizing sorbent zones." Examples of both methods are provided in Example I of the specification where avidin was used as an analyte binding partner. Specifically, for non-covalently immobilization, avidin was printed onto a substrate. For covalently immorbilization, avidin was derivatized with a photolabile linker moiety, printed onto a substrate, and exposed to a UV light for cross-linking. See page 17, lines 18-30. Although it was found that non-covalently immobilized avidin is less desirable for analyte mass analysis compared to covalently immobilized avidin, this comparison does not imply that non-covalently immobilized analyte binding partner in general cannot be used for analyte mass analysis.

In fact, the specification provides two examples (Examples II and III) where analyte binding partners were non-covalently immobilized to a substrate. For instance, in Example III, biotinylated monoclonal antibodies (analyte binding partners) were immobilized to a substrate by jet printing. No covalent bonding was involved. Human myeloma proteins (analytes) were added to form analyte – analyte binding partner complexes. The complexes were recognized by dye-labeled second antibodies to the analytes. See page 22, lines 16-26. It was found that the fluorescence from the dye responded to the analyte mass (FIG. 10).

Therefore, the specification provides not only general guidelines for non-covalent immobilization of analyte binding partners but also specific examples of using non-covalently immobilized analyte binding partners for analyte mass analysis. A skilled artisan would be able to follow these teachings to achieve mass-sensing analysis using non-covalently immobilized analyte binding partners.

In sum, a reasonable amount of general guidance and a sufficient number of specific examples are given in the specification with respect to the method of determining analyte mass by measuring fluorescence, the assay conditions, and the non-covalent immobilization of analyte binding partners. Merely routine, if any, experimentation would be required to practice the claimed invention. Thus, Applicants submit that claims 1-22, 26-30, 32-34, 37-38, and 41-42 are enabled by the specification in their full scope and that the rejection should be withdrawn.

Claim Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 23-25, 31, 35-36, and 39-40 are rejected as being incomplete for omitting essential structural cooperative relationships of elements. See the Office Action, page 8, part 6. More specifically, the Examiner asserted that there is no structural relationship between the claimed binding array and sample binding used on the array. Applicants respectfully traverse.

Among the rejected claims, claims 23 and 35 are independent claims. Claim 23 is discussed in detail below as an example.

Claim 23, as previously presented, is directed to an analyte binding array for harvesting analyte from a liquid sample. The array comprises a plurality of microscopic sorbent zones immobilized on a surface of a substrate, wherein a microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner, the matrix extending up to 200 nm vertically from the surface of the substrate, the analyte binding partner being present in an amount sufficient to substantially deplete the analyte from a sample and concentrate the analyte on the microscopic sorbent zone, the microscopic zone being from about 60 to about 500 μm in diameter and the sample containing about 10^5 to about 10^{10} molecules of analyte per 100 μl of the sample, wherein a volume of the sample is from 20 to 500 μl.

Contrary to the Examiner's assertion, there is clear structural relationship between the claimed binding array and the sample to be analyzed on the array. The binding array comprises a plurality of microscopic sorbent zones, and each microscopic sorbent zone comprises a multi-layer matrix of an analyte binding partner. The sample to be analyzed on the array contains an analyte. The structural relationship between the binding array and the sample is that the analyte binding partner on the array and the analyte in the sample are capable of binding to each other (page 9, lines 4-7 of the specification). However, for the sole purpose of expediting the prosecution of this application, Applicants have amended claim 23 such that it now recites "an analyte binding partner <u>that binds an analyte from a sample</u>." Claim 35 has been similarly amended.

Applicants submit that, claims 23 and 35, as amended, recite essential structural cooperative relationships of elements. Claims 24-25, 31, 36, and 39-40, dependent directly or indirectly from claim 23 or 35, are also complete. The rejection should be withdrawn.

Claim Rejection Under 35 U.S.C. § 102

Claims 23-25, 35-36, and 39-40 are rejected as being anticipated by Ekins (EP 0 304 202 B1; "'202"). See the Office Action, page 9, part 7. More specifically, the Examiner asserted that the '202 patent teaches an analyte binding array

comprising a plurality of microscopic sorbent zones having the claimed amount of an analyte binding partner and zone diameter, as well as the claimed sample size. Applicants respectfully traverse.

Among the rejected claims, claims 23 and 35 are independent claims. Claims 23 and 35 are directed to an analyte binding array for harvesting analyte from a liquid sample. The array comprises a plurality of microscopic sorbent zones immobilized on a surface of a substrate, each sorbent zone having a diameter from 60 μm to 500 μm.

Contrary to the Examiner's assertion, the '202 patent does not teach a sorbent zone with a diameter of 60-500 um (equivalent to an area of 0.003-0.2 mm^2). Instead, the '202 patent states in general that the sizes of the spots are less than 10 mm^2 and preferably less than 1 mm^2 (page 6, lines 4-5), and uses 1 mm^2 as an example (page 5, lines 56-57 and page 7, line 53).

In this connection, Applicants point out that, when a claim is directed to a narrow range while the reference teaches a broad range, and there is evidence of unexpected results within the claimed narrow range, it may be reasonably conclude that the narrow range is not disclosed with sufficient specificity in the reference to constitute an anticipation of the claim. The unexpected results may also render the claim unobvious. See MPEP § 2131.03.

The narrow range recited in claims 23 and 35 are not anticipated or rendered obvious by the '202 patent because there is evidence of unexpected results within the claimed narrow range. Claims 23 and 35 are drawn to an analyte binding array for use in a mass-sensing binding assay with high sensitivity for very low quantities of analyte. The mass-sensing binding assay requires maximizing analyte-binding capacity, and in the mean time, minimizing the microscopic sorbent zone (page 10, line 19 – page 11, line 10) such that an analyte in a test sample is depleted by an analyte binding partner in the microscopic sorbent zone. By limiting the sorbent zone to a diameter of 60-500 um, unexpected results were achieved:

(a) In Example I of the specification, about 10^{10} molecules of avidin were immobilized in a sorbent zone, resulting in a three-dimensional matrix about 3

monolayers deep (see page 10, last paragraph – page 12, first paragraph of the Amendment dated March 25, 2005). As discussed in Section 7 of the Declaration dated May 13, 2002 ("Declaration"), those skilled in the art would not have expected that such a three-dimensional matrix is possible without "blocking" analytes' access to their binding partners on the lower layers.

(b) In the present invention, microscopic sorbent zones in contact with conventional sample volumes were capable of harvesting substantially the same amount of an analyte from the sample within the same incubation time as the equivalent amount of the analyte binding partner spreads across a microtiter plate well or distributed in the sample solution. As explained in Section 8 of the Declaration, such a result was unexpected given the complexities of the non-equilibrium conditions, cooperativity in binding, diffusion, local concentration effects, and other nonlinear effects occurring when an analyte is concentrated onto the microscopic sorbent zones.

(c) As explained at page 7, lines 21-31 of the specification, because the present assay concentrates substantially all analyte present in a defined sample volume, the same amount of analyte can be harvested, for example, by the array from 100 μl of 10^{-13} M analyte solution and from 10 μl of 10^{-12} M analyte solution. Accordingly, the fluorescence readings generated by analyte bound to the sobent zone will be the same in both cases. By sensing mass instead of concentration, the instant assay unexpectedly increases sensitivity of the analyte detection.

(d) As explained in Section 9 of the Declaration, in the present invention, substantially the same signal as in the conventional microplate assay was generated in a zone about 100 times smaller than that of a microwell. Since background signals are roughly proportional to the viewed surface area, the microscale assay of the present invention advantageously and unexpectedly produced a signal-to-background ratio orders of magnitude greater than conventional microplate assays.

Furthermore, contrary to the teachings of the present invention that analyte-binding capacity should be maximized while the microscopic sorbent zone should be

minimized, the '202 patent emphasizes that "the use of high quantities of binding agent is neither necessary for good sensitivity in immunoassays nor is it generally desirable" and that "the amount of binding agent is reduced so that only an insignificant proportion of the analyte is reversibly bound to it, generally less than 10%, usually less than 5% and for optimum results only 1 or 2% or less" (page 3, lines 28-32). Thus, the '202 patent teaches against increasing coating density of the analyte binding partner and depletion of the analyte by the analyte binding partner. As such, the '202 patent would not have motivated one skilled in the art to search for a narrow range below 1 mm^2 in which the microscopic sorbent zone is minimized without losing the maximal analyte-binding capacity required for depleting an analyte from a sample.

To conclude, claims 23 and 35 are neither anticipated nor rendered obvious by the '202 patent because the '202 patent does not specifically disclose a sorbent zone having a diameter of 60-500 um and unexpected results derived from the claimed narrow diameter range. Claims 23 and 35 are also unobvious in view of the '202 patent because the '202 patent does not provide motivation for a skilled artisan to identify the claimed narrow diameter range. By the same token, claims 24-25, 36, and 39-40, dependent directly or indirectly from claim 23 or 35, are not anticipated or rendered obvious by the '202 patent, either. Applicants respectfully request that the rejection be withdrawn.

Applicants believe that the application, as amended, is in condition for allowance and early, favorable action is respectfully solicited.

If for any reason the Examiner finds the application other than in condition for allowance, the Examiner is requested to call the undersigned attorney at the Los Angeles telephone number (310) 789-5100 to discuss the steps necessary for placing the application in condition for allowance.

If there are any fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 50-1314.

Respectfully submitted,

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